AD_____

Award Number: W81XWH-05-1-0608

TITLE: Properties of Leukemia Stem Cells in a Novel Model of Cml Progression to Lymphoid Blast Crisis

PRINCIPAL INVESTIGATOR: Craig T. Jordan

CONTRACTING ORGANIZATION: University of Rochester
Rochester, NY 14642

REPORT DATE: October 2007

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
01-10-2007	Final	15 SEP 2005 - 14 SEP 2007

4. TITLE AND SUBTITLE

Properties of Leukemia Stem Cells in a Novel Model of Cml Progression to Lymphoid Blast Crisis

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-05-1-0608

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Craig T. Jordan

E-Mail: craig_jordan@urmc.rochester.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of Rochester
Rochester, NY 14627-0140

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
Background: Progression of CML from chronic phase to lymphoid blast crisis is a poorly characterized event. However, at least some of the molecular events that accompany evolution of the disease have been described. One such event, mutation of the p16Ink4a/p19Arf locus, is known to occur in approximately 50% of patients developing acute lymphoid disease. Based on this observation, we generated a novel mouse model in which combination of the well-known BCR/ABL translocation with loss of function mutation at the p16Ink4a/p19Arf locus induces a very robust and authentic lymphoid blast crisis. In order to understand the earliest origins of disease pathogenesis, we have used the model to characterize leukemia stem cells (LSC) as they progress from chronic phase disease to blast crisis. Intriguingly, in the chronic phase, LSC can be derived only by introduction of BCR/ABL into normal hematopoietic stem cells (HSC). Expression of BCR/ABL at later stages of hematopoietic differentiation does not support development of disease. In contrast, upon loss of p16Ink4a/p19Arf activity, expression of BCR/ABL is sufficient to induce disease at multiple stages of hematopoietic differentiation (HSC, CLP, Pro-B, etc). These findings indicate evolution of LSC during progression to lymphoid blast crisis can occur via mutations in several different types of stem and progenitor cells, an observation that has important ramifications for the clinical management of patients with lymphoid, as compared to myeloid blast crisis.
Objective/Hypothesis: We hypothesize that mutations occurring during various stages of hematopoietic development will generate a heterogeneous population of LSC in lymphoid blast crisis, and that the various classes of LSC will differ with regard to their drug sensitivity. The objective of the proposal is to characterize discrete LSC types and define methods for their eradication.

15. SUBJECT TERMS
No subject terms provided.

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
					USAMRMC
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			**19b. TELEPHONE NUMBER** *(include area code)*
U	U	U	UU	9	

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

Table of Contents

Properties of leukemia stem cells in a novel model of CML progression to lymphoid blast crisis (CM050044)

Introduction

This report describes progress to date for the above referenced DOD grant. The objective of the study was to employ a novel mouse model of CML blast crisis to characterize various forms of leukemia stem cells (LSC) and their relative properties. In particular, distinguishing how differing normal target cells contribute to disease pathogenesis was regarded as an important priority in establishing relative heterogeneity of LSC.

Body

Task 1: To determine the biological properties of BCR/ABL+ LSC arising via mutation at different stages of lymphoid differentiation (Months 1-6):

We have completed a detailed series of studies on the developmental origin of LSC using retroviral gene transfer into various stem or progenitor cell populations. The data show:

1) Expression of BCR/ABL in HSC of Arf null mice creates a mixed lineage leukemia in which both acute lymphoid and myeloproliferative populations are evident. In the majority of animals, the lymphoid disease ultimately expands and is lethal. The data indicate that while both lymphoid and myeloid developmental fates are possible, the lymphoid disease is more aggressive. The frequency of leukemia-initiating cells in this model is approximately 1 in 2000 cells (see figures 1-2 below).
2) Expression of BCR/ABL in CLP or ProB populations from Arf null mice only creates an acute lymphoid disease (Figure 2). The frequency of leukemia-initiating cells in this model is approximately 1 in 200 cells. The immunophenotype of bulk tumor is consistent with a pre-B cell ALL. The immunophenotype of leukemia-initiating cells is Sca-1+, VLA-5+ (Figures 3-4), as assessed by in vitro colony-forming ability.

Task 2. To determine the relative drug sensitivity of LSC that arise in distinct stem/progenitor populations (Months 6-12):

The primary objective of these studies was to determine whether the drug treatment is cytotoxic to LSC in the above referenced model. The drugs evaluated were imatinib and the parthenolide analog DMAPT (dimethylaminoparthenolide). Treatment of LSC arising at different stages of development with imatinib showed no differences (data not shown), suggesting that all forms of LSC are equally resistant to this type of drug. In contrast, treatment of varying LSC with DMAPT showed strong cytotoxicity (up to 90% cell death in vitro). However, again, we detected no

differences between LSC arising at different developmental stages. While very limited with respect to the types of drugs tested, these findings do not support our original hypothesis, which proposed that LSC from differing developmental origins may display variable drug sensitivity.

Key research accomplishment

Validation of the BCR/ABL + Arf null model as a system in which LSC pathogenesis and drug sensitivity can be studied.

Reportable outcomes

Overview of experimental design

Enriched cells from Ink4a/Arf-null (exon 2 & 3 deletion) Arf-null (exon 1β deletion) or wild type C57B6 mice were transduced with retroviral particles to express p210BCR/ABL and GFP. Enriched cells include: hematopoietic stem cells (HSC), common lymphoid progenitors (CLP), ProB, and PreB populations. The cells were then transplanted into cohort of sub-lethally irradiated wild type recipients through tail vein injection. 10 to 14 days post transplantation, peripheral blood of primary recipients were collected to evaluate possible disease progression via analyzing the morphology and amount of total white blood cells (WBC), percentage and lineage marker of GFP positive cells.

Fig. 1

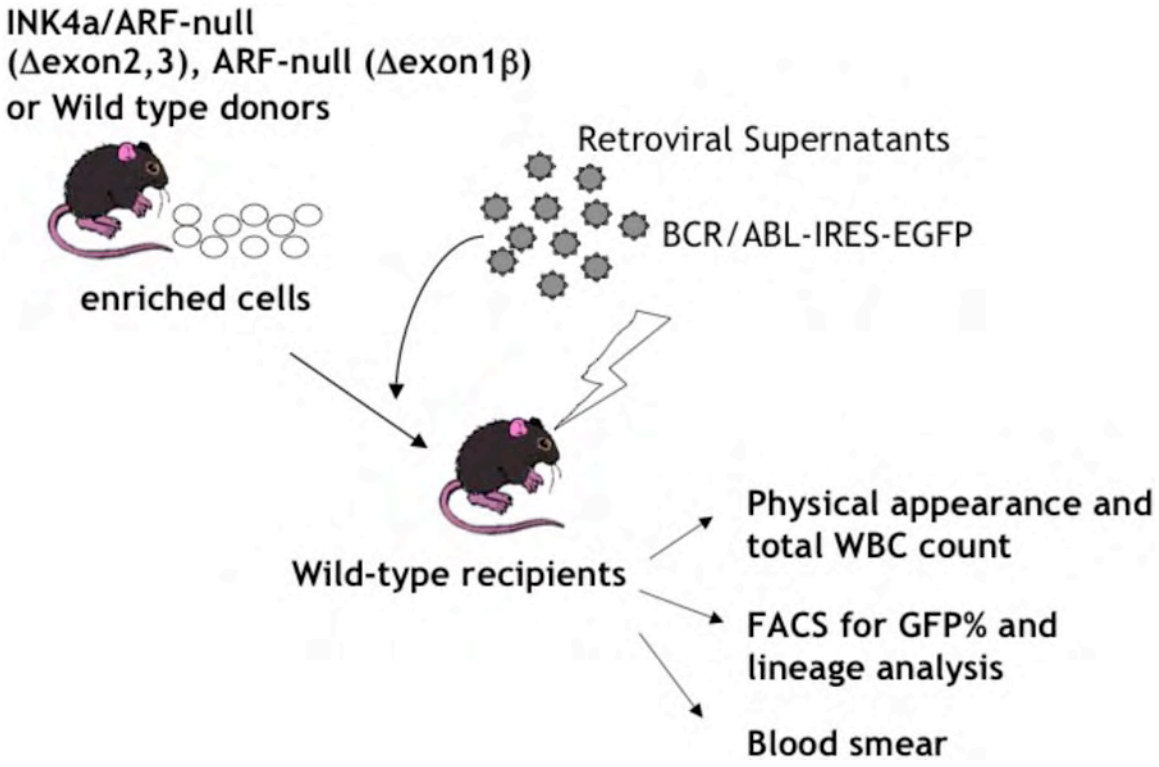
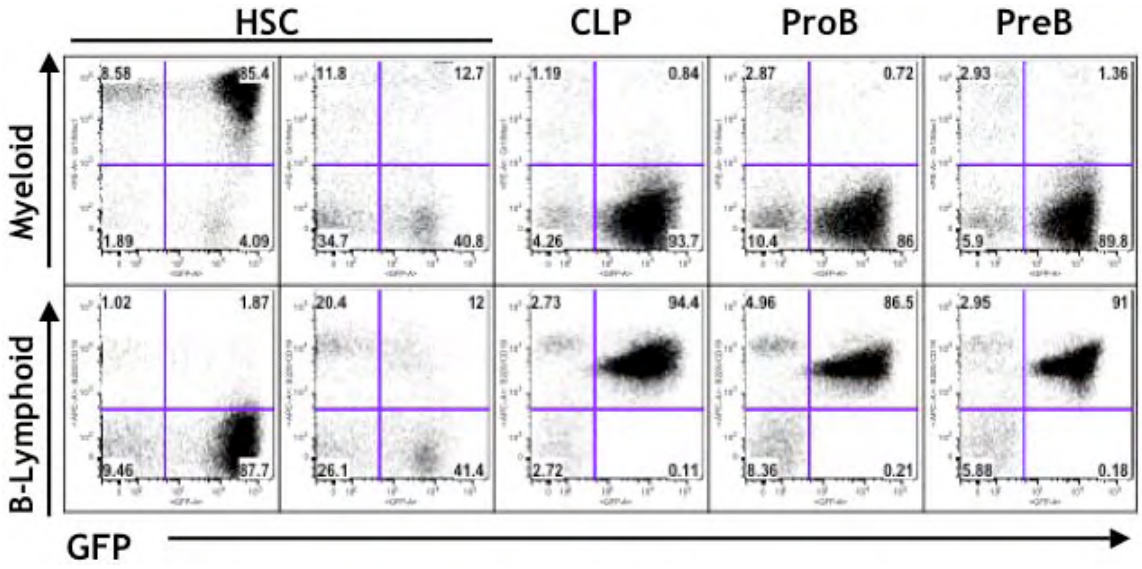


Figure 2

Phenotypic analysis of animals transplanted with retrovirally transduced HSC, CLP, ProB or PreB cells from Ink/Arf null mice. The myeloid vs. lymphoid nature of the disease varies as a function of developmental origin.

A.



B.

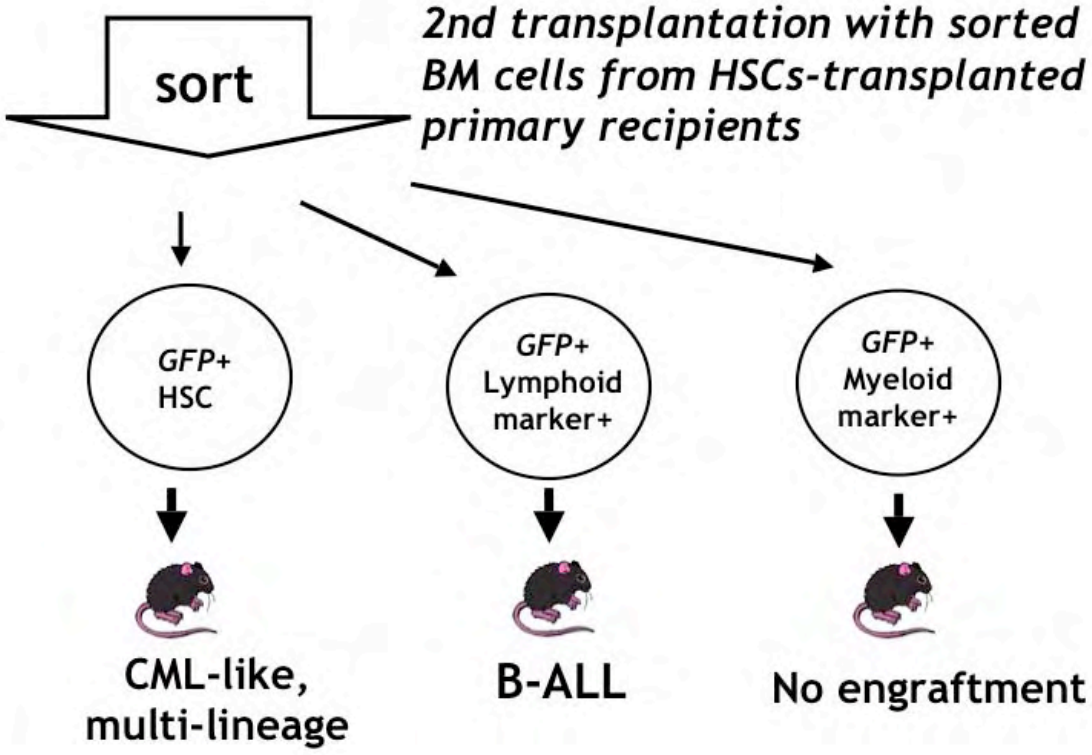
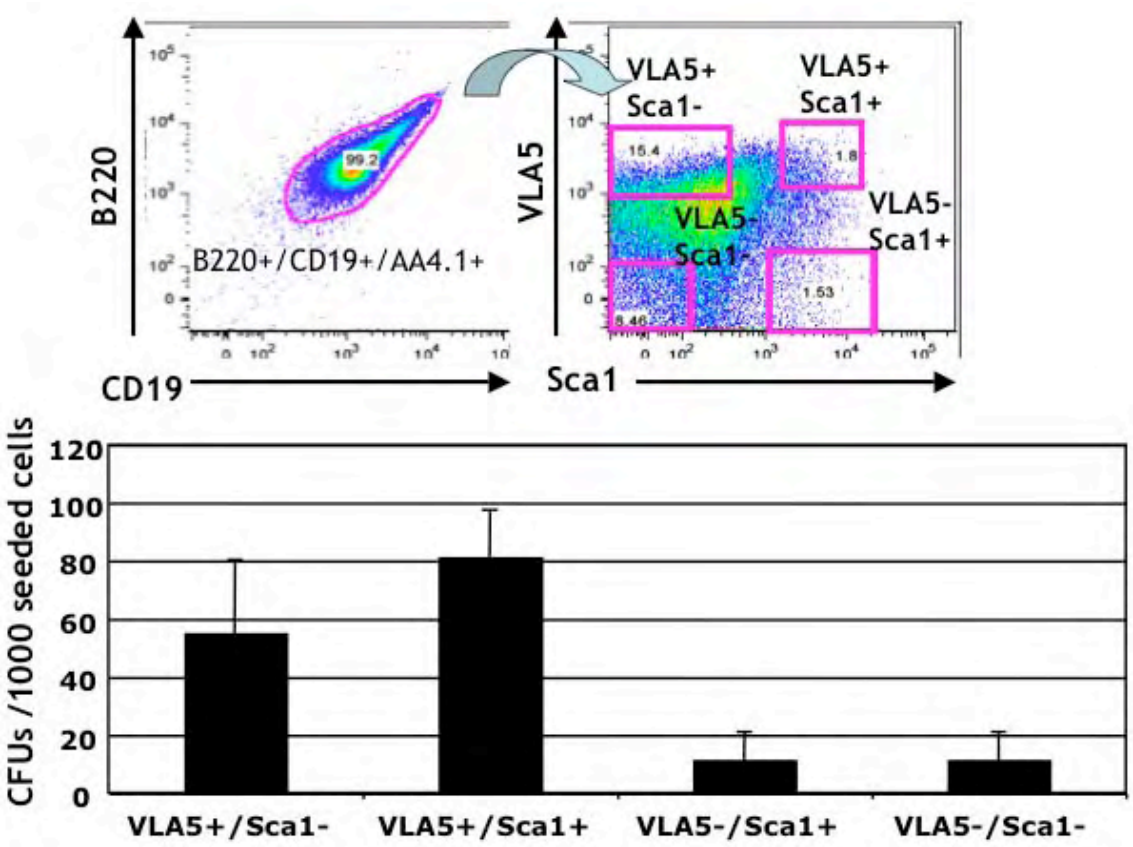


Figure 3

BM cells from B-ALL recipients were stained with surface antigens: B220, CD19, Sca1 and VLA5. GFP positive B cells were selected and further sorted into 4 different groups (VLA5+Sca1-, VLA5+Sca1+, VLA5-/Sca1+, and VLA5-/Sca1-), which were each assayed for colony-forming unit (CFU) ability.



Conclusions

In wild type mice, only BCR/ABL mutation at the stem cell level is sufficient to induce disease, and such disease is almost always myeloid. In contrast, these studies demonstrate that loss of Arf function is a critical determinant defining the nature and prevalence of LSCs. In the absence of Arf, LSC can arise from multiple developmental stages (HSC, CLP, ProB, PreB). Moreover, the type of disease can vary as a function of the particular developmental origin, with most manifestations being lymphoid. These findings indicate that loss of Arf function is sufficient to induce the transition from CML to lymphoid blast crisis. Further, the data suggest that the developmental stage at which blast crisis evolution occurs can vary. We speculate that acute disease arising from varying developmental stages may display differing biological properties. However, in the studies performed for this project, differences in drug sensitivity amongst varying forms of LSC was not observed. The studies outlined above provide a model system in which these theories may be further explored and new therapeutic options may be evaluated.

References

N/A

Appendices

N/A